**Table 4:** Univariate and multivariate predictors of haemorrhagic events in all late Fontan survivors

| | 5 years | | | 10 years | | | 15 years | | |
|---|---|---|---|---|---|---|---|---|---|
| | Haemorrhagic ($n$ = 13) | | | Haemorrhagic ($n$ = 9) | | | Haemorrhagic ($n$ = 6) | | |
| | HR | 95% Confidence interval | $P$ value | HR | 95% Confidence interval | $P$ value | HR | 95% Confidence interval | $P$ value |
| Univariate analyses | | | | | | | | | |
| Patient characteristics | | | | | | | | | |
| Age at first Fontan (years) | 1.06 | 0.98-1.11 | 0.1153 | 1.05 | 0.94-1.12 | 0.2537 | 1.24 | 1.04-1.46 | **0.0194** |
| Male | 8.98 | 1.76-164 | **0.0048** | 4.83 | 0.88-89.6 | 0.0729 | 1.60 | 0.31-11.6 | 0.5840 |
| Non-LV SV | 2.18 | 0.66-9.76 | 0.2094 | 4.56 | 0.83-84.8 | 0.0866 | 2.89 | 0.47-55.3 | 0.2815 |
| Heterotaxy syndrome | 6.08 | 1.81-27.4 | **0.0030** | 16.40 | 2.99-304 | **0.0005** | 10.30 | 1.64-197 | **0.0109** |
| Type of repair | | | | | | | | | |
| APC vs TCPC | 0.88 | 0.12-3.80 | 0.8818 | - | - | - | 0.84 | 0.04-5.40 | 0.8736 |
| Fenestration | 3.20 | 0.48-12.9 | 0.1958 | 5.20 | 0.73-24.9 | 0.0897 | 5.41 | 0.27-42.6 | 0.2138 |
| Medications | | | | | | | | | |
| Wafarin | 4.30 | 1.26-13.6 | **0.0215** | 2.41 | 0.35-10.5 | 0.3206 | - | - | - |
| Antiplatelet | 0.57 | 0.18-1.75 | 0.4631 | 1.03 | 0.27-4.18 | 0.9640 | 0.27 | 0.04-1.38 | 0.1153 |
| Diuretics | 2.29 | 0.75-7.64 | 0.1445 | 3.99 | 1.05-18.9 | **0.0422** | 1.10 | 0.15-5.62 | 0.9165 |
| ACEI/ARB | 7.15 | 1.96-25.1 | **0.0040** | 6.45 | 1.57-31.7 | **0.0105** | 3.18 | 0.62-23.1 | 0.1674 |
| β-Blocker | 2.10 | 0.11-11.6 | 0.5254 | 4.41 | 0.93-16.8 | 0.0606 | 1.77 | 0.25-9.07 | 0.5258 |
| Antiarrhythmic/PMI | 8.36 | 2.40-27.3 | **0.0016** | 4.36 | 0.91-16.8 | 0.0640 | 3.05 | 0.42-15.9 | 0.2359 |
| Haemodynamics | | | | | | | | | |
| CVP (per 1 mmHg) | 1.08 | 0.87-1.34 | 0.4842 | 1.16 | 0.91-1.49 | 0.2204 | 0.86 | 0.57-1.27 | 0.4519 |
| PA index (per 10 $mm^2/m^2$) | 0.97 | 0.90-1.03 | 0.4212 | 0.98 | 0.89-1.05 | 0.4947 | 0.98 | 0.87-1.09 | 0.7713 |
| EF (per 1%) | 0.96 | 0.91-1.01 | 0.1380 | 1.01 | 0.94-1.07 | 0.8144 | 0.98 | 0.91-1.07 | 0.6932 |
| Cardiac index (per 1.0 l/min/$m^2$) | 0.69 | 0.28-1.44 | 0.3545 | 1.80 | 0.84-3.29 | 0.1240 | 1.95 | 0.73-4.25 | 0.1640 |
| Arterial oxygen saturation (per 1%) | 0.88 | 0.80-1.00 | **0.0434** | 0.88 | 0.83-0.94 | **0.0016** | 0.90 | 0.79-1.14 | 0.2987 |
| Multivariate analyses | | | | | | | | | |
| Male | 8.80 | 1.36-182 | **0.0192** | - | - | - | - | - | - |
| Heterotaxy syndrome | 4.96 | 1.22-26.0 | **0.0244** | 20.30 | 2.09-589 | **0.0063** | *6.66* | *0.84-138* | *0.0740* |
| Arterial oxygen saturation (per 1%) | - | - | - | 0.86 | 0.73-1.00 | **0.0472** | - | - | - |

Bold values indicates statistically significant. Italic values indicates a tendency of statistical significance on the multivariate analysis.
Abbreviations are the same as in Tables 1, 2 and 3.

haemoptysis with late Fontan operation may be due to the development of aortopulmonary collateral arteries during a longer hypoxic pre-Fontan period and/or palliative procedures. Next to infection, congenital heart disease is one of the most common causes of haemoptysis in children [20] and life-threatening haemoptysis after Fontan operation has been reported [21]. Thus, after the Fontan operation, we need to think of haemorrhagic as well as thrombo-embolic events especially in patients with heterotaxy syndrome. The reason for the high incidence of haemorrhagic events in patients with heterotaxy syndrome is not apparent from the present study. Intestinal microvascular malformation was demonstrated in one adolescent patient with this syndrome who experienced recurrent intestinal bleeding [22], implying an unknown systemic microangiopathy. This issue might be one of our study subjects in the future. In addition, varicose veins of the lower extremity may not be uncommon late after the Fontan operation [23] and may cause bleeding, and surgical management may be required in some patients.

Several studies have shown that overall coagulation homoeostasis might be as well-balanced in clinically stable Fontan patients as in those with end-stage liver disease [11, 24], implying that some pathological stress, such as infection, heart failure or haemodynamic deterioration due to arrhythmia, may destroy the delicate balance, leading to haemostatic events. Unstable haemodynamics immediately after the Fontan operation and liver dysfunction, haemodynamic deterioration and arrhythmias remote from the time of the Fontan operation may explain the two high-prevalence phases of haemostatic events depicted in the present and previous studies [4]. In addition, inappropriate anticoagulation may have adversely impacted hematological events [4]. In fact, among patients with heart failure, a reduced risk of ischaemic stroke with warfarin was offset by an increased risk of major haemorrhage [25]. Therefore, in our current era, 'timely' rather than stereotyped, 'time-limited' anticoagulant strategy may prevent thrombo-embolic events when Fontan patients experience stress conditions, such as surgery, infection and other unexpected complications.

Finally, the anticoagulant regimens employed varied widely and this situation was similar to a previous report [16]. We have been modifying medications, including anticoagulation, if necessary, based on our routine comprehensive follow-up evaluations. So, when the patient has acceptable haemodynamics with few risk factors for thrombo-embolic events, we now discontinue warfarin therapy to minimize the risk of haemorrhagic events. On the other hand, 25 patients (7%) of the 367 6-month survivors restarted their warfarin therapy after a transient warfarin-free period; those patients were categorized as W4 in this study, implying a difficulty in standardization of anticoagulation. A large-scale and long-term randomized, controlled trial based on the individual plasma profile-guided therapy may be required to standardize haemostatic management.

Our study has several limitations. First, because of its retrospective nature, it could not uncover cause and effect relationships.

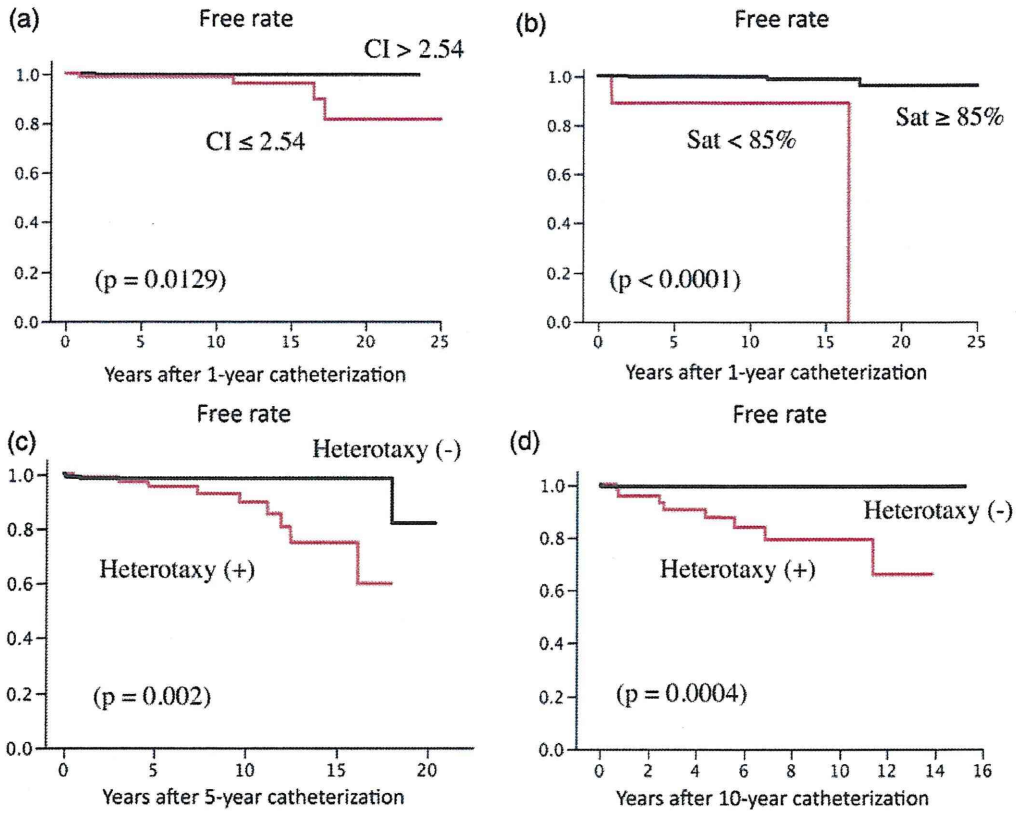


**Figure 3:** Kaplan-Meier thrombo-embolic event-free rate curves of those with cardiac index (CI) ≤2.54 l/min/m$^2$ and CI >2.54 l/min/m$^2$ and the curves of those with and without low arterial oxygen saturation [(Sat) <85%] based on the 1-year post-Fontan status are shown in (**a**) and (**b**), respectively. The haemorrhagic event-free rate curves of those with and without heterotaxy syndrome are shown in (**c**) and (**d**), respectively.

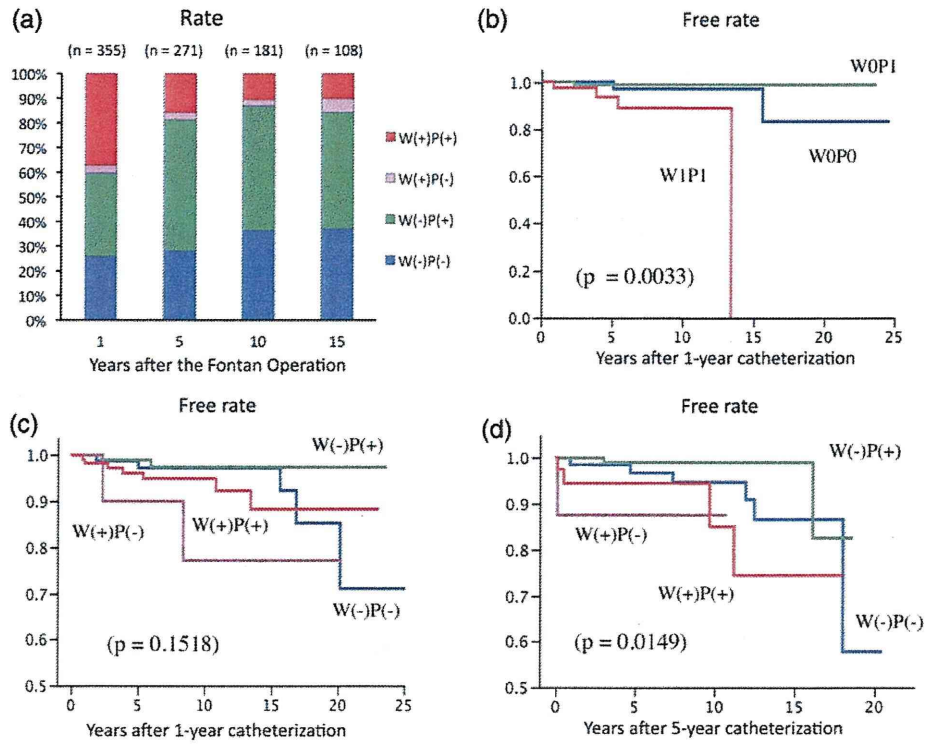


**Figure 4:** Four combinations of anticoagulation regimens with warfarin and antiplatelets at 1-, 5-, 10- and 15-year postoperative phases (**a**). The groups of W(+)P(+), W(+)P(−), W(−)P(+) and W(−)P(−) indicate patients with anticoagulant therapy with warfarin and antiplatelets, those with warfarin, those with antiplatelet and those without any anticoagulant, respectively. The Kaplan-Meier haemorrhagic event-free rate curves have been constructed in the major three sub-groups with fixed anticoagulant regimens (**b**) and the curves of the four sub-groups according to the four combinations of anticoagulant regimens at 1- and 5-year postoperative phases (**c** and **d**, respectively).

Second, although we focused on symptomatic events, we may have underestimated the prevalence of thrombo-embolic events as the prevalence depended on the method of evaluation, and this was a further limitation due to its retrospective nature. We could not identify clinically relevant determinants of late thrombo-embolic events because of the small number of events. Third, our anticoagulation strategy was not standardized even when we decided on the optimal individual regimen(s) at our clinical conference. In addition, international normalized ratios were not analysed in this study. Fourth, we evaluated the impact of pre- and postoperative Fontan haemodynamics on haemostatic events on the assumption that each hazard was constant during the entire follow-up period; however, there may have been some significant haemodynamic changes with the corresponding hazard ratio over time that could have influenced the haemostatic events. Lastly, our results may not be generalized because, in addition to our post-Fontan routine-scheduled comprehensive assessment, our Fontan strategy is somewhat unique, such as off-pump Fontan operation and early establishment of Fontan circulation at 1–2 years of age.

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# Abnormalities in blood coagulation, fibrinolysis, and platelet activation in adult patients after the Fontan procedure

Lidia Tomkiewicz-Pajak, MD, PhD,[a,b] Piotr Hoffman, MD, PhD,[c] Olga Trojnarska, MD, PhD,[d] Magdalena Lipczyńska, MD, PhD,[c] Piotr Podolec, MD, PhD,[a,b] and Anetta Undas, MD, PhD[a,b]

**Background:** Thrombosis occurs in up to 30% of patients with various forms of congenital single ventricle after the Fontan procedure. We investigated hemostatic abnormalities in adult Fontan patients.

**Methods:** Forty-eight Fontan patients between ages 18 and 40 years, including 10 (21%) patients with previous thromboembolism 5 to 15 years after surgery, and 35 control subjects matched for age and sex were studied. Coagulation factors and inhibitors, together with markers of fibrinolysis, platelets, and endothelial activation, were determined in peripheral venous blood plasma.

**Results:** Compared with control subjects, Fontan patients showed lower, although mostly within normal ranges, values of all coagulation factors, as well as reduced free protein S, in association with higher antithrombin and free tissue factor pathway inhibitor levels. Thrombin generation, reflected by prothrombin fragment 1.2, and platelet activation markers were increased in Fontan patients. The plasma clot lysis time was prolonged in Fontan patients, which was associated with an increased activity of thrombin-activatable fibrinolysis inhibitor. Fontan patients with previous thromboembolism had lower oxygen saturation, coagulation factors V and VIII, and free protein S, and increased von Willebrand factor, soluble CD40 ligand, and P-selectin. Other laboratory or clinical parameters were not associated with prior thrombotic episodes.

**Conclusions:** Adult Fontan patients are characterized by enhanced platelet activation and endothelial injury, heightened thrombin formation, and impaired fibrinolysis. Patients showed reduced free protein S levels, increased platelet activation, and endothelial damage after thromboembolic events observed late after Fontan surgery. Our findings indicate novel prothrombotic mechanisms in adult Fontan patients, which might help to optimize thromboprophylaxis. (J Thorac Cardiovasc Surg 2014;147:1284-90)



Fontan surgery has become the treatment of choice in patients with single-ventricle physiology. The purpose of this procedure is to normalize volume load of the ventricle and separate the pulmonary and systemic circulation to achieve normal or near-normal levels of blood oxygen. Several modifications of the Fontan surgery can provide excellent palliation for patients with various forms of single ventricles.[1] Long-term follow-up evaluation of Fontan patients showed an 85% survival rate.[2,3] The longer the time since the procedure, the more frequently remote complications occur. In addition, thrombosis occurs in 8% to 33% of Fontan patients.[2-5] Thrombi can be detected by echocardiography in the systemic venous pathway, systemic ventricle, or ligated pulmonary artery. Asymptomatic pulmonary emboli have been reported in 17% of Fontan patients.[6] Thrombosis risk factors reported in Fontan patients involve arrhythmias, slow venous flow, increased central venous pressure, cyanosis, ventricular dysfunction, liver injury, and effusion protein-losing enteropathy.[5,7] A high prevalence of chronic venous insufficiency in adult Fontan patients also has been reported.[8]

Studies on alterations of blood coagulation after Fontan surgery conducted in children showed lower plasma levels of coagulation factor (F) V, VII, and VIII, along with low protein C, free protein S, and plasminogen levels.[5,9-12] Mechanisms underlying thromboembolism in Fontan patients are unclear. Odegard et al[13] showed that an increase in FVIII after Fontan surgery and a decrease in protein C levels were associated with a high risk of thrombosis in 37 patients (age, ≤49 mo) with hypoplastic left heart syndrome from stage I through Fontan completion. In Fontan patients ages 7 to 26 years, increased von Willebrand factor (vWF) levels have been observed, suggesting endothelial damage.[14] It has been reported that efficiency of fibrinolysis is preserved in those patients based on normal plasma tissue-type plasminogen activator and plasminogen activator inhibitor-1 (PAI-1). We performed a comprehensive analysis

From the Institute of Cardiology,[a] Jagiellonian University Medical College, and John Paul II Hospital,[b] Krakow, Poland; Department of Adult Congenital Heart Diseases,[c] Institute of Cardiology, Warsaw, Poland; and 1st Department of Cardiology,[d] University of Medical Sciences, Poznan, Poland.

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Address for reprints: Anetta Undas, MD, PhD, Institute of Cardiology, Jagiellonian University Medical College, 80 Pradnicka St, 31-202 Krakow, Poland (E-mail: mmundas@cyf-kr.edu.pl).

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**Abbreviations and Acronyms**
CLT = clot lysis time
ELISA = enzyme-linked immunosorbent assay
F = factor
PAI-1 = plasminogen activator inhibitor-1
$Sao_2$ = oxygen saturation
TAFI = thrombin activatable fibrinolysis inhibitor
TFPI = tissue factor pathway inhibitor
vWF = von Willebrand factor

of blood coagulation, fibrinolysis, and platelet activation in adult Fontan patients. We investigated hemostatic abnormalities observed in adult Fontan patients to identify those associated with thromboembolism after the procedure.

## PATIENTS AND METHODS

### Study Participants

Forty-eight white Fontan patients (26 men, 22 women) ages 18 to 40 years and 35 apparently healthy control subjects matched for age and sex were included in the study. They were recruited between January 2011 and December 2011. The exclusion criteria were as follows: any acute illness, cancer, renal insufficiency (creatinine level >120 μmol/L), diabetes mellitus, an acute vascular event, alcohol abuse, or pregnancy.

Echocardiography was performed in all the patients and single-ventricle systolic function was assessed.[10] Oxygen saturation ($SaO_2$) was measured by pulse oximetry.

The diagnosis of deep vein thrombosis was established by a positive finding of color duplex sonography. The diagnosis of pulmonary embolism was based on the presence of typical symptoms and positive results of high-resolution spiral computed tomography. Stroke was diagnosed based on the World Health Organization criteria. In Fontan patients with previous thromboembolic events, thrombophilia screening including FV Leiden, 20210A prothrombin mutation, deficiencies in natural anticoagulants and antiphospholipid antibodies was performed.

The University Ethical Committee approved the study and patients provided written informed consent.

### Laboratory Investigations

Fasting blood samples were collected into 0.1 volume of 3.2% trisodium citrate from the antecubital vein with minimal stasis on the same day that clinical data were recorded. In anticoagulated patients, blood was drawn at least 5 days after anticoagulation withdrawal. Citrated blood samples were centrifuged at 3000 g for 20 minutes and stored in aliquots at –80°C until further use. Red blood cell count, white blood cell count, platelet count, hematocrit level, total protein level, alanine aminotransferase level, creatinine level, C-reactive protein level, and international normalized ratio were assayed by routine laboratory techniques.

### Coagulation Proteins

Fibrinogen was determined using the thrombin clotting time method. Prothrombin and coagulation factors (F)V, FVII, FVIII, FIX, and FX were measured by 1-stage clotting assays using factor-deficient plasmas (Siemens, Marburg, Germany). Antithrombin activity was measured using Berichrom (Siemens). Free (ie, active) tissue factor pathway inhibitor (free TFPI) was determined by an enzyme-linked immunosorbent assay (ELISA) (Diagnostica Stago, Asnieres, France). Protein C activity was measured using a chromogenic substrate assay (Siemens). Free protein S was determined using a latex ligand immunoassay (Instrumentation Laboratory, Milan, Italy). The immunoenzymatic assay was used to determine plasma prothrombin fragments 1.2 (F1.2; Siemens).

### Fibrinolytic Proteins

Plasma antiplasmin and plasminogen were measured by chromogenic assays (STA Stachrom 2 antiplasmin and STA Stachrom plasminogen; Diagnostica Stago). Plasma PAI-1 antigen levels were measured by an ELISA (American Diagnostica, Greenwich, Conn). Thrombin activatable fibrinolysis inhibitor (TAFI) antigen was determined with an ELISA (Chromogenix, Lexington, Mass). Plasma TAFI activity was measured by a chromogenic assay using the Actichrome Plasma TAFI Activity Kit (American Diagnostica). Soluble thrombomodulin was measured by an ELISA (Diagnostica Stago).

Fibrin clot lysis time (CLT) was measured as described.[15] Briefly, citrated plasma was mixed with 15 mmol/L calcium chloride, 10,000-diluted human tissue factor (Innovin; Dade Behring), 12 μmol/L phospholipid vesicles, and 60 ng/mL recombinant tissue-type plasminogen activator (Boehringer Ingelheim, Ingelheim, Germany). Turbidity of this mixture was measured at 405 nm at 37°C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents clot formation, to the midpoint of the maximum-turbid-to-clear transition, representing the lysis of a clot.

### Platelet and Endothelial Cell Activation

vWF antigen (Diagnostica Stago), and 2 platelet activation markers, that is, soluble CD40 ligand (sCD40L; R&D Systems, Indianapolis, Ind), and P-selectin (R&D Systems) also were measured in plasma.

All the hemostatic measurements were performed by technicians blinded to the sample status. The coefficients of intra-assay and interassay variations were less than 9%.

### Statistical Analysis

Data are expressed as the mean ± SD for normally distributed variables or as the median (interquartile range) for non-normally distributed variables. The Kolmogorov-Smirnov test was used to assess conformity with a normal distribution. Categoric values were analyzed using the $\chi^2$ test. Continuous variables were compared by the Student $t$ test when distributed normally or by the Mann-Whitney $U$ test for non-normally distributed variables. Correlations between the individual parameters were calculated using the Pearson or Spearman rank correlation as appropriate. All clinical and laboratory variables that showed an association with thrombosis in the univariate model ($P < .05$) and did not show substantial correlations ($r \geq 0.4$) with another independent variable were included in the stepwise multiple logistic regression analysis. The statistical analyses were performed with the Statistica 9.0 software (StatSoft, Tulsa, Okla).

The study was powered to have a 90% chance of detecting a 10% difference in vWF using a $P$ value of .001, based on the values of vWF in the previous article.[14] To show a difference of this magnitude or greater, 28 patients were required in each group.

## RESULTS

The majority of patients had the left ventricular type of single ventricle and they were in New York Heart Association class I or II (Table 1). The Fontan patients had a lower white blood cell and platelet counts, and a higher red blood cell count, hemoglobin level, alanine aminotransferase level, creatinine level, and international normalized ratio compared with the controls (Table 2).

### Coagulation Factors and Inhibitors

Compared with the controls, Fontan patients had lower values (from 19% to 35%) of all coagulation factors and

**TABLE 1. Patient characteristics**

| Variable | Fontan patients (n = 48) |
|---|---|
| Age at Fontan surgery (y) | 5 (1-17) |
| Postoperative time (y) | 18 (3-30) |
| Anatomic diagnosis, n (%) | |
| Tricuspid atresia | 20 (42) |
| VSD, pulmonary atresia | 9 (18.5) |
| Hypoplasia of right ventricle | 10 (21) |
| Double-outlet right ventricle with hypoplastic left ventricle | 9 (18.5) |
| Ventricle type, n (%) | |
| Right ventricle | 9 (19) |
| Left ventricle | 39 (81) |
| Type of Fontan, n (%) | |
| Atriopulmonary connection | 16 (33.3) |
| Total cavopulmonary connection | 29 (60.4) |
| Modified Fontan | 3 (6.3) |
| Fenestration, n (%) | 11 (23) |
| Systemic ventricle function, n (%) | |
| Good | 25 (52) |
| Fair | 16 (33) |
| Decreased | 5 (11) |
| Poor | 2 (4) |
| NYHA functional class, n (%) | |
| I | 14 (29) |
| II | 32 (67) |
| III | 2 (4) |
| IV | 0 |
| Cardiac rhythm, n (%) | |
| Sinus rhythm | 42 (88) |
| Atrial fibrillation | 4 (8) |
| Pacemaker | 1 (2) |
| Medication use, n (%) | |
| Warfarin | 10 (21) |
| Aspirin | 16 (33) |
| Prednisone | 3 (6) |
| Mean oxygen $SaO_2$ on room air (%) | 92 (90-97) |

Data are presented as median (interquartile range) or number (%). *VSD*, Ventricular septal defect; *NYHA*, New York Heart Association; *$SaO_2$*, oxygen saturation.

**TABLE 2. Comparison of Fontan patients and controls**

| Variable | Controls (n = 36) | Fontan patients (n = 48) | *P* value |
|---|---|---|---|
| Age at enrollment (y) | 27 (19-40) | 25 (18-40) | .06 |
| Male, n (%) | 19 (57) | 26 (54) | .23 |
| BMI ($kg/m^2$) | 22 ± 3 | 22 ± 3 | .43 |
| WBC ($10^3/\mu L$) | 6.1 ± 1.4 | 5.5 ± 1.6 | .023 |
| RBC ($10^9/\mu L$) | 4.9 [0.3] | 5.2 [0.9] | .001 |
| Hematocrit (%) | 46 ± 3 | 46 ± 3 | .84 |
| Platelet count ($10^3/\mu L$) | 226 ± 36 | 148 ± 48 | <.001 |
| ALT (IU/L) | 24 ± 9 | 29 ± 12 | .016 |
| Total protein (g/dL) | 73 [5] | 74 [11] | .36 |
| Creatinine ($\mu mol/L$) | 69 ± 11 | 74 ± 15 | <.001 |
| CRP (mg/L) | 1.6 [1.6] | 1.6 [3.7] | .99 |
| INR | 0.97 [0.2] | 1.1 [0.2] | .003 |

Data are presented as mean ± SD, median [interquartile range], or number (%). *BMI*, Body mass index; *WBC*, white blood cell; *RBC*, red blood cell; *ALT*, alanine aminotransferase; *CRP*, C-reactive protein; *INR*, international normalized ratio.

free protein S, however, most of the values were within normal ranges (Table 3). Interestingly, the patients had higher antithrombin and free TFPI levels, whereas the protein C level was similar in both groups. $SaO_2$ correlated positively with prothrombin (r = 0.38; $P$ = .009), FV (r = 0.49; $P$ = .001), FVIII (r = 0.32; $P$ = .03), and FX (r = 0.31; $P$ = .03), and free protein S (r = −0.4; $P$ = .005). Compared with the controls, Fontan patients showed higher thrombin generation, reflected by increased prothrombin fragments F1.2. There was a positive correlation between F1.2 and age (r = 0.32; $P$ = .03), but not with $SaO_2$ or clinical variables (data not shown).

### Fibrinolysis

The Fontan patients had higher PAI-1 antigen levels than the controls, however PAI activity was similar in both groups (Table 3). Interestingly, TAFI activity was higher by 24% ($P$ < .001) in the patients despite comparable levels of TAFI antigen (Table 3). A global fibrinolysis marker, CLT, was 16% ($P$ < .001) longer in the Fontan patients than in the controls.

CLT in the Fontan patients showed positive associations with PAI-1 antigen (r = 0.71; $P$ < .001), TAFI activity (r = 0.69; $P$ < .001), and prothrombin fragments F1.2 (r = 0.74; $P$ < .001). TAFI activity correlated with PAI-1 activity (r = −0.6; $P$ < .001), PAI- 1 antigen (r = 0.58; $P$ < .001), and F1.2 (r = 0.41; $P$ = .004).

### Platelet and Endothelial Activation Markers

sP-selectin and sCD40L were higher by 92% and 25%, respectively, in Fontan patients. We also found vWF to be 23% higher in Fontan patients (Table 3). There were inverse correlations between sP-selectin and $SaO_2$ (r = −0.52; $P$ < .001), prothrombin (r = −0.35; $P$ = .01), FV (r = −0.4; $P$ = .005), FVIII (r = −0.33; $P$ = .02), FIX (r = −0.37;, $P$ = .01), FX (r = −0.38; $P$ = .009), and free protein S (r = −0.48; $P$ = .001).

None of the clinical characteristics in the Fontan patients showed associations with fibrinolytic, platelet, or endothelial markers (data not shown).

### Fontan Patients With Thrombosis

Previous arterial or venous thromboembolism occurred in 10 (21%) patients 5 to 15 years (median, 12 y) after the Fontan surgery. Three patients developed thrombi in the Fontan circulation, 4 patients had previous deep vein thrombosis, 1 patient had an isolated pulmonary embolism, and, finally, 2 patients had both previous ischemic stroke and deep vein thrombosis. Only 1 patient with a prior thromboembolism had thrombophilia (ie, heterozygous FV Leiden mutation).

**TABLE 3. Coagulation factors/inhibitors, fibrinolysis variables, and platelet and endothelial activation markers in Fontan patients and controls**

| Variable | Controls (n = 36) | Fontan patients (n = 48) | *P* value |
|---|---|---|---|
| Coagulation factors and inhibitors | | | |
| FII (%) | 109 ± 16 | 87 ± 17 | <.001 |
| FV (%) | 104 ± 15 | 73 ± 24 | <.001 |
| FVII (%) | 102 [14] | 82 [18] | <.001 |
| FVIII (%) | 132 ± 28 | 108 ± 29 | .001 |
| FIX (%) | 114 ± 20 | 75 ± 20 | <.001 |
| FX (%) | 115 ± 21 | 87 ± 24 | <.001 |
| Fibrinogen (g/L) | 3.2 ± 1.3 | 2.4 ± 0.5 | .005 |
| Prothrombin fragments F1.2 (pmol/L) | 185 [31] | 263 [74] | <.001 |
| Antithrombin (%) | 99 ± 14 | 109 ± 13 | .001 |
| Free TFPI (ng/mL) | 17 [4] | 25 [9] | <.001 |
| Protein C (%) | 105 [18] | 103 [23] | .42 |
| Free protein S (%) | 95 [13] | 85 [13] | <.001 |
| Fibrinolysis variables | | | |
| $\alpha_2$AP (%) | 102 ± 9 | 100 ± 12 | .29 |
| Plasminogen (%) | 102 ± 10 | 104 ± 12 | .67 |
| PAI-1 activity (IU/mL) | 9 ± 1.1 | 9 ± 3 | .28 |
| PAI-1 antigen (ng/mL) | 14 ± 4 | 26 ± 6 | <.001 |
| TAFI activity ($\mu$g/mL) | 25 ± 4 | 31 ± 8 | <.001 |
| TAFI antigen (%) | 106 ± 14 | 104 ± 11 | .72 |
| CLT (min) | 80 ± 12 | 93 ± 14 | <.001 |
| Platelet and endothelial activation markers | | | |
| vWF (%) | 122 ± 20 | 150 ± 28 | <.001 |
| sP-selectin (ng/mL) | 142 ± 29 | 273 ± 69 | <.001 |
| sCD40L (pg/mL) | 361 ± 76 | 450 ± 156 | .006 |
| TM (ng/mL) | 3.9 [2.2] | 3.0 [2.9] | .1 |

Data are presented as mean ± SD or median [interquartile range]. *$\alpha_2$AP*, Antiplasmin; *CLT*, clot lysis time; *sCD40L*, soluble CD40 ligand; *TM*, thrombomodulin; *F*, factor; *TFPI*, free tissue factor pathway inhibitor; *PAI-1*, plasminogen activator inhibitor-1; *TAFI*, thrombin activatable fibrinolysis inhibitor; *vWF*, von Willebrand factor; *sP-selectin*, soluble P-selectin.

The Fontan patients with a history of thrombosis had a lower $SaO_2$. Moreover, lower values of FV, FVIII, and free protein S were observed. Interestingly, increased vWF, sCD40L, and P-selectin were found in these patients (Table 4). Other clinical and hemostatic parameters were not affected by a history of thrombosis. The multivariate model showed that free protein S and sCD40L were the only independent predictors of thromboembolic events late after Fontan surgery ($\chi^2 = 30.7$, $P < .001$; Table 5).

## DISCUSSION

We present a comprehensive evaluation of blood coagulation, fibrinolysis, platelet, and endothelial cell activation in adult patients who underwent the Fontan procedure in their childhood or adolescence. The major findings of our study were uniformly decreased coagulation factors in adult Fontan patients with a paradoxically increased thrombin generation in the circulating blood, probably owing to enhanced platelet activation and endothelial injury coupled with a slightly lower inhibition of thrombin formation by reduced free protein S.

Our study clearly indicates that there are complex hemostatic disturbances in adult Fontan patients. On the one hand, they seem to favor bleeding by reduction of coagulation factor production, and on the other hand they promote thrombosis through reduced protein S, hypofibrinolysis, impaired endothelial function, and increased platelet activation. However, the net effect of all the hemostatic disturbances in Fontan patients tilts the balance toward prothrombotic tendency.

Of note, we observed different patterns of hemostatic alterations in adult Fontan patients as compared with those reported previously in children mostly in the early postoperative period. In adult patients late after the Fontan surgery, we found significant reductions in coagulation factors including FVIII (all the individual alterations were mild and did not exceed the normal ranges). However, bleeding is fairly uncommon in Fontan patients, which suggests that despite a lower platelet count and coagulation factors, other potent mechanisms can overcome these alterations, leading to a prothrombotic state as evidenced by heightened thrombin generation and reflected by increased F1.2 levels. Interestingly, there were significantly increased levels of 2 coagulation inhibitors, liver-derived antithrombin and endothelium-derived free TFPI, in the Fontan patients as compared with controls. It might be hypothesized that this phenomenon is a compensatory mechanism aiming at restoring the hemostatic balance when larger amounts of thrombin are generated.

Of note, our study shows an impaired fibrinolytic potential in adult Fontan patients and hypofibrinolysis is

**TABLE 4. Comparison between Fontan patients without and with thrombosis**

| Variable | Patients without thrombosis (n = 38) | Patients with thrombosis (n = 10) | *P* value |
|---|---|---|---|
| Age at enrollment, (y) | 24 (18-40) | 27 (18-36) | .18 |
| Age at surgery, (y) | 6.9 (1-16) | 5.6 (2-17) | .19 |
| Postoperative time, (y) | 17 (3-30) | 21 (14-27) | .09 |
| Male, n (%) | 20 (53) | 6 (60) | .68 |
| Anatomic diagnosis, n (%) | | | .2 |
| Tricuspid atesia | 16 (42) | 4 (40) | |
| VSD, pulmonary atesia | 6 (16) | 4 (40) | |
| Hypoplasia of right ventricle | 7 (18) | 2 (20) | |
| Double-outlet right ventricle with hypoplasia of left ventricle | 9 (24) | 0 (0) | |
| Ventricle type, n (%) | | | .07 |
| Right ventricle | 9 (24) | 0 (0) | |
| Left ventricle | 29 (76) | 10 (100) | |
| Type of Fontan, n (%) | | | .2 |
| Atriopulmonary connection | 16 (42) | 0 (0) | |
| Total cavopulmonary connection | 19 (50) | 10 (100) | |
| Modified Fontan | 3 (8) | 0 (0) | |
| Systemic ventricle function, n (%) | | | .13 |
| Good | 21 (55) | 4 (40) | |
| Fair | 13 (34) | 3 (30) | |
| Decreased | 2 (6) | 3 (30) | |
| Poor | 2 (5) | 0 (0) | |
| NYHA functional class, n (%) | | | .49 |
| I | 12 (31) | 2 (20) | |
| II | 25 (66) | 7 (70) | |
| III | 1 (3) | 1 (10) | |
| IV | 0 | 0 | |
| $SaO_2$ (%) | 92 [6] | 84 [8] | .003 |
| Platelet count ($10^3/\mu L$) | 142 [49] | 152 [44] | .79 |
| Hematocrit (%) | 44 ± 7 | 49 ± 3 | .07 |
| Prothrombin (%) | 89 ± 15 | 79 ± 22 | .1 |
| FV (%) | 82 [25] | 61 [20] | .058 |
| FVII (%) | 82 [19] | 80 [15] | .64 |
| FVIII (%) | 113 [28] | 82 [26] | .03 |
| FIX (%) | 75 [20] | 67 [22] | .23 |
| FX (%) | 89 ± 25 | 80 ± 18 | .28 |
| Antithrombin (%) | 110 ± 12 | 105 ± 15 | .22 |
| Protein C (%) | 104 ± 26 | 102 ± 28 | .85 |
| Free protein S (%) | 89 ± 10 | 70 ± 9 | <.001 |
| PAI-1 antigen (ng/mL) | 26 ± 6 | 25 ± 6 | .67 |
| PAI-1 activity (IU/mL) | 9 ± 3 | 9 ± 3 | .65 |
| TAFI antigen (%) | 104 ± 11 | 104 ± 12 | .93 |
| TAFI activity (ug/mL) | 31 ± 8 | 32 ± 9 | .69 |
| CLT (min) | 91 [15] | 89 [12] | .34 |
| Prothrombin fragments F1.2 (pmol/L) | 261 [76] | 275 [73] | .88 |
| Plasminogen (%) | 104 ± 11 | 100 ± 15 | .3 |
| $\alpha_2$AP (%) | 99 ± 12 | 103 ± 11 | .45 |
| vWF (%) | 144 ± 23 | 174 ± 33 | .002 |
| sP-selectin (ng/mL) | 251 ± 55 | 353 ± 18 | <.001 |
| sCD40L (pg/mL) | 417 ± 134 | 579 ± 173 | .003 |

Data are presented as mean ± SD, median [interquartile range], or number (%). *VSD*, Ventricular septal defect; *NYHA*, New York Heart Association; *TAFI*, thrombin activatable fibrinolysis inhibitor; *$\alpha_2$AP*, antiplasmin; *sCD40L*, soluble CD40 ligand; *$SaO_2$*, oxygen saturation; *F*, factor; *PAI-1*, plasminogen activator inhibitor-1; *CLT*, clot lysis time; *vWF*, von Willebrand factor; *sP-selectin*, soluble P-selectin.

driven mostly by increased TAFI activity. Increasing evidence indicates that increased TAFI levels occur in venous thromboembolism and ischemic stroke.[16,17] High TAFI activity in Fontan patients is a novel finding and it might contribute to late thromboembolism in this population. To assess fibrinolysis efficiency, we used the

**TABLE 5. Univariate and multivariate predictors for thrombosis in Fontan patients**

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| Variable | Odds ratio (95% CI) | *P* value | Odds ratio (95% CI) | *P* value |
| $SaO_2$ (%) | 0.87 (0.78-0.97) | .015 | | |
| Free protein S (%) | 0.81 (0.71-0.93) | .003 | 0.77 (0.63-0.94) | .012 |
| P-selectin (ng/mL) | 15.0 (2.6-85.2) | .003 | | |
| vWF (%) | 1.04 (1.01-1.07) | .014 | | |
| sCD40L (pg/mL) | 1.89 (1.16-3.09) | .012 | 2.71 (1.08-6.82) | .034 |
| Factor VIII (%) | 0.97 (0.94-0.99) | .049 | | |

*CI*, Confidence interval; *sCD40L*, soluble CD40 ligand; *vWF*, von Willebrand factor; *$SaO_2$*, oxygen saturation.

plasma-based clot lysis assay, in which coagulation is initiated by a tissue factor without addition of human thrombin, and tissue-type plasminogen activator is used.[18] In our study, CLT was prolonged in adult Fontan patients and correlated positively with PAI-1 antigen, TAFI activity, and F1.2. It remains to be established whether CLT might help identify subjects at increased risk of thromboembolic events among those patients.

In adult Fontan patients, nonpulsative flow in the pulmonary circulation, slow venous flow, and hypoxemia may damage endothelial cells. We observed evidence for endothelial injury in Fontan patients as reflected by increased plasma vWF levels. It has been postulated that vWF is a marker of endothelial dysfunction in contrast to thrombomodulin, which increases subsequent cell membrane injury.[19,20]

Unexpectedly, we observed that Fontan patients with prior thromboembolism had lower FV and FVIII, both of which are associated with $SaO_2$, and free protein S. Cheung et al[21] reported that systemic oxygen blood saturation correlated positively with plasma levels of anticoagulant and procoagulant factors, and those abnormalities preceded the Fontan procedure with a tendency to normalize after the surgery in association with improved systemic oxygenation.

A decrease of $SaO_2$ in adults after Fontan surgery may result from a right-to-left shunt through an atrial fenestration or venovenous collaterals. Atz et al[22] observed lower rest $SaO_2$ without an increased risk of thrombosis during an 8-year follow-up period in Fontan patients with fenestration. In our study there were no differences in the prevalence of fenestration between the patients with and without a history of thrombosis. Desaturation also could reflect a progressive decrease in single-ventricle competence and an increase in pulmonary pressure.[1,2,7] This mechanism is likely to cause lower $SaO_2$ in our patients.

A novel finding of the present study was increased platelet activation, as evidenced by higher sP-selectin and sCD40L in Fontan patients who experienced thromboembolic events. P-selectin, largely derived from activated platelets, is considered a marker of inflammation or platelet activation, but it also can induce procoagulant activity through increased tissue factor expression.[23] Despite significantly reduced coagulation factor levels, it is likely that by providing a catalytic surface for prothrombinase complex formation, in combination with reduced levels of free protein S, activated platelets largely contribute to a prothrombotic tendency in Fontan patients. Of note, none of the patients with previous thromboembolism was taking aspirin at the time of blood collection, which might have resulted in increased platelet activation. There is evidence that low-dose aspirin is not potent enough to reduce circulating levels of platelet-derived proteins significantly (eg, sCD40L[24]).

Several limitations of the study should be acknowledged. First, the number of patients in the study was small and the patients were heterogeneous with respect to cardiac diagnosis and the Fontan surgery type. However, our group is representative of the current growing adult Fontan patient population in the real-life clinical practice. Second, the true incidence of thrombosis may have been underestimated because we analyzed only symptomatic events. Because patients were studied post hoc after thromboembolic events, we cannot exclude that the abnormalities observed in this subset are to some extent secondary to thrombosis. However, given a long period of time that elapsed since the event, it is unlikely that the abnormalities are solely the result of past thromboembolism. Coagulation factors and inhibitors were not determined at the time of the index event, and it is known that transient increases in coagulation factors, particularly FVIII, and decreases in natural anticoagulants, especially antithrombin and protein C, are observed commonly during acute thrombosis. Therefore, changes during acute thrombosis are perceived as a poor predictor of overall function of the hemostatic system in human beings.

In conclusion, we found that adult Fontan patients are characterized by specific abnormalities in blood coagulation, enhanced platelet activation, and endothelial injury, associated with heightened thrombin formation and impaired fibrinolysis. Patients show reduced free protein S, increased platelet activation, and endothelial damage after thromboembolic events observed late after Fontan surgery. Further studies are needed to evaluate the potential benefits from combined antiplatelet and anticoagulant thromboprophylaxis in Fontan patients.

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Clinical Research

# A Multicenter, Randomized Trial Comparing Heparin/Warfarin and Acetylsalicylic Acid as Primary Thromboprophylaxis for 2 Years After the Fontan Procedure in Children

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Paul Monagle, MD, MSc, MBBS[*], Andrew Cochrane, MD[*], Robin Roberts, MSc[†], Cedric Manlhiot, BSc[‡], Robert Weintraub, MBBS[*], Barbara Szechtman, BA[†], Marina Hughes, DPhil[§], Maureen Andrew, MD[‡], Brian W. McCrindle, MD, MPH[‡], , Fontan Anticoagulation Study Group



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## Objectives

The purpose of this study was to compare the safety and efficacy of acetylsalicylic acid (ASA) and warfarin for thromboprophylaxis after the Fontan procedure.

## Background

Fontan surgery is the definitive palliation for children with single-ventricle physiology. Thrombosis is an important complication; the optimal thromboprophylaxis strategy has not been determined.

## Methods

We performed a multicenter international randomized trial of primary prophylactic anticoagulation after Fontan surgery. Patients were randomized to receive for 2 years either ASA (5 mg/kg/day, no heparin phase) or warfarin (started within 24 h of heparin lead-in; target international normalized ratio: 2.0 to 3.0). Primary endpoint (intention to treat) was thrombosis, intracardiac or embolic (all events adjudicated). At 3 months and 2 years after the Fontan procedure, transthoracic and transesophageal echocardiograms were obtained as routine surveillance. Major bleeding and death were primary adverse outcomes.

## Results

A total of 111 eligible patients were randomized (57 to ASA, 54 to heparin/warfarin). Baseline characteristics for each group were similar. There were 2 deaths unrelated to thrombosis or bleeding. There were 13 thromboses in the heparin/warfarin group (3 clinical, 10 routine echo) and 12 thromboses in the ASA group (4 clinical, 8 routine echo). Overall freedom from thrombosis 2 years after Fontan surgery was 19%, despite thrombosis prophylaxis. Cumulative risk of thrombosis was persistent but varying and similar for both groups ($p = 0.45$). Major bleeding occurred in 1 patient in each group.

## Conclusions

There was no significant difference between ASA and heparin/warfarin as primary

thromboprophylaxis in the first 2 years after Fontan surgery. The thrombosis rate was suboptimal for both regimens, suggesting alternative approaches should be considered. (International Multi Centre Randomized Clinical Trial Of Anticoagulation In Children Following Fontan Procedures; NCT00182104)

## Key Words

anticoagulation; Fontan procedure; pediatrics; thrombosis

## Abbreviations and Acronyms

ASA, acetylsalicylic acid; INR, international normalized ratio; TEE, transesophageal echocardiography

Fontan surgery, first performed for tricuspid atresia in the late 1960s, has evolved as the definitive palliative surgery for all children with univentricular cardiac physiology, irrespective of whether they have a functioning right, left, or indeterminate ventricle. The Fontan principle is diversion of systemic venous return directly to the pulmonary arteries, and the use of the single ventricle as a functioning systemic ventricle (1). In subsequent years, many modifications of the original procedure have been described, although the basic principle remains the same (2, 3 and 4). Improvements in surgical techniques and supportive care have significantly improved long-term survival for children after Fontan surgery; hence, the frequency of Fontan surgery continues to increase (5, 6 and 7).

Thrombosis remains a major complication after Fontan surgery, presenting as intracardiac or intravascular thrombosis, cerebrovascular thromboembolism, or other embolic phenomena. The true frequency of thrombembolism post-Fontan surgery remains unknown (1 and 8). Cross-sectional surveys using transesophageal echocardiography (TEE) reported a prevalence of intracardiac thrombosis of 17% to 33% (9, 10 and 11). Cohort studies that had venous thrombosis or arterial emboli (or both) as a primary outcome measure reported incidences of venous thrombosis ranging from 3% to 19% and the incidence of stroke ranging from 3% to 19%, depending on the nature of the cohort and the duration and nature of follow-up. The reported mortality from post-Fontan surgery thromboembolism is 25% (1). In this context, a multitude of primary thromboprophylaxis strategies have been suggested. However, there have been no prospective data on which to base any prophylactic protocols (12 and 13).

We performed a multicenter, randomized, controlled trial of primary prophylactic anticoagulation after Fontan surgery, comparing 2 commonly used strategies, to determine their efficacy and safety in the first 2 years after Fontan surgery.

## Methods

### Study subjects

All patients scheduled for a Fontan procedure were eligible for inclusion. Exclusion criteria included the presence of a recognized indication for long-term anticoagulation; characteristics associated with an increased risk of bleeding; known medical contraindication to heparin, warfarin, or acetylsalicylic acid (ASA); inability to supervise therapy due to social or geographic reasons; and pregnancy or potential pregnancy during the study period.

### Randomization

Randomization was performed centrally immediately after completion of the Fontan procedure. Randomization was stratified by center.

### Study intervention

The study interventions were delivered in accordance with standard protocols for a 2-year period. Subjects randomized to warfarin received heparin initially at a dose of 10 to 20 U/kg/h. Usually within 24 h of starting heparin and when the patient was first able to tolerate oral medication, a loading dose of warfarin of 0.1 mg/kg was given. The dose was then titrated to achieve a target international normalized ratio (INR) of 2.0 to 3.0, and heparin was discontinued when the INR first reached 2.0. Adjustment of the maintenance dose of warfarin was dependent on INR monitoring as follows: INR of 2.0 to 3.0, no change in dose; INR of 1.1 to 1.4, increase dose by 20%; INR of 1.5 to 1.9,

increase dose by 10%; INR of 3.1 to 3.5, decrease dose by 10%; and INR of >3.5, hold warfarin until INR was <3.5, then decrease dose by 20% when restarted. Patients randomized to ASA were started when they were tolerating any oral intake (ASA, 5 mg/kg/day, no heparin phase, no monitoring). Because Fontan patients are well documented to be at an important risk of thrombotic complications, there was believed to be insufficient clinical equipoise to include a nonintervention group.

### Measurements

Study measurements included a baseline medical record review to abstract data regarding demographics, underlying cardiac anatomy, previous interventions and complications, and previous and current medical therapy. Data regarding the Fontan procedure and post-operative complications were collected. Clinical monitoring was performed at 3, 6, 12, 18, and 24 months after randomization and whenever it was clinically indicated. INR monitoring and dose adjustments for warfarin to maintain the patient within the therapeutic range were the responsibility of the treating center and were dependent on the clinical status of the child. INR monitoring was prescribed to be performed at least every 2 to 3 weeks for stable patients and more frequently for patients with dosing challenges. Routine transthoracic echocardiography and TEE was performed at 3 and 24 months post-Fontan surgery. All patients were requested to attend all study visits regardless of whether they were still taking their assigned study medication and/or had reached a study outcome. Thrombosis with clinical presentation, severe bleeding complications, Fontan takedown, death, and all other serious adverse events were captured for all patients.

### Blinding

All medication use was open label. Local echocardiographic assessments were performed by sonographers and echocardiographers in a blinded manner. There was an independent central adjudication of clinically driven and routine echocardiograms. All thrombosis and major adverse clinical events were adjudicated by an expert panel.

### Study endpoints

The primary endpoint was any thrombotic event (venous or arterial). This was defined as the ultrasound appearance of a space-occupying lesion within the cardiovascular system (mild laminar thickening of the internal surface of the Fontan pathway was not included) or the occurrence of a clinical event known to be strongly associated with thrombus (cardioembolic stroke, pulmonary embolism). Additional endpoints included Fontan takedown, death, and study drug discontinuation or crossover.

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# Thromboembolism and the Role of Anticoagulation in the Fontan Patient

M. L. Jacobs · K. K. Pourmoghadam

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**Abstract** Among factors contributing to morbidity and failure of the Fontan circulation is the group of events referred to as thromboembolic complications. These events have been variously attributed to low flow states, stasis in the venous pathways, right-to-left shunts, blind cul-de-sacs, prosthetic material, atrial arrhythmias, and hypercoagulable states. Numerous investigations, most retrospective, have been undertaken to characterize thromboembolic events; describe the frequency and circumstances of these occurrences; and relate the risk of these events to patient, surgical, hemodynamic, and hematologic factors. Practices vary widely with respect to strategies of prophylactic anticoagulation in the hopes of minimizing the occurrence and morbidity of thromboembolism after Fontan operations. Review of the literature suggests that the factors associated with thromboembolic events after Fontan operations likely represent a complex field of biologic factors with multiple interactions. It is unlikely that a single agent will represent the solution to this complex problem.

Although the trend in operative mortality for modified Fontan procedures has been one of steady diminution from mortality rates approaching 30% in the earliest series to mortality rates less than 5% in contemporary reports, some factors contributing to morbidity and mortality seem to persist. One of the major factors contributing to morbidity and failure of the Fontan circulation is the group of events referred to as thromboembolic complications.

Thromboembolic complications in Fontan patients fall into two major categories [44]. The first involves thrombosis within the surgically created pathways between the vena cavae and the pulmonary arteries. These pathways are frequently referred to as the Fontan pathway or Fontan circuit. Thrombus formation within these pathways can cause local obstruction to flow with adverse hemodynamic consequences and may also embolize or extend into the pulmonary arteries [2, 20, 25, 34, 47, 57]. Also, in the presence of either intentional (surgically created) or incidental right-to-left intracardiac communications, thrombus in the systemic venous pathway or compartment may embolize through "fenestrations" to the systemic arterial circulation [28, 49, 51]. The second major category of thromboembolic events in Fontan patients involves thrombus originating in the pulmonary venous pathway or compartment (e.g., on the pulmonary venous side of a baffle) or in the systemic ventricle or ligated main pulmonary artery stump [37, 40, 48, 53]. In these cases, morbidity occurs principally in the form of embolism to the central nervous system [42, 62], the coronary circulation [49, 62], or, less commonly, elsewhere in the systemic circulation.

Numerous hypotheses have been invoked to explain the troubling frequency of thromboembolic events in Fontan patients. Thromboembolism after Fontan operations has been variously attributed to low flow states, stasis in the venous pathways, right-to-left shunts, blind cul-de-sacs, prosthetic materials, atrial arrhythmias, and hypercoagulable states. Numerous investigations, most retrospective, have been undertaken to characterize thromboembolic events; describe the frequency and circumstances of these occurrences; and relate the risk of thromboembolic events

M. L. Jacobs (✉)
Section of Cardiothoracic Surgery, St. Christopher's Hospital for Children, Erie Avenue at Front Street, Philadelphia, PA 19134, USA
e-mail: Marshall.Jacobs@tenethealth.com

K. K. Pourmoghadam
Pediatric Cardiac Surgery,
Geisinger Medical Center, Danville, PA 17822, USA

to patient, surgical, hemodynamic, and hematologic factors. A wide variety of practices have evolved with respect to various strategies of prophylactic anticoagulation in the hopes of minimizing the occurrence and morbidity of thromboembolism after Fontan operations. Although numerous authors tout their hypotheses, and the preferred anticoagulation strategies of institutions or individual practitioners, efforts toward evidence-based practice in this area are very limited.

In 2002, Monagle and Karl [45] published an article that included the work product of a comprehensive MEDLINE literature search of the English language literature from 1971 to 2000 using the following key words: Fontan, univentricular heart, children, thrombosis, congenital heart disease, cavopulmonary, and palliation. They identified and analyzed 51 studies and gleaned information concerning incidence, potential morbidity and mortality, risk factors, prophylactic options, and risk/benefit ratio of prophylactic anticoagulation as related to thromboembolic events after Fontan surgery. They concluded that, at the time, there was insufficient data to make clear recommendations about optimal anticoagulant prophylaxis.

Not included among the 51 reports analyzed in Monagle and Karl's review was our cohort study [30], also published in 2002, in which low-dose aspirin prophylaxis (81 mg/day) was used consistently and exclusively as a prophylactic anticoagulation regimen in 72 consecutive patients undergoing Fontan operations during a 5-year period. The patients had been monitored prospectively, with thromboembolism as the primary outcome measure. There were no early or late deaths. Follow-up was complete with 2882 patient-months at a mean of 40 months. There were no documented thromboembolic events: All suspicious events were investigated by transesophageal echocardiography and brain imaging. There were no hemorrhagic events or aspirin-related complications. This study was unique at the time because of the consistent use of a single anticoagulation strategy in a cohort of patients followed prospectively with evaluation for thromboembolic events as a primary end point. With the goal of shedding more light on this complex subject, we updated the literature search originally performed by Monagle and Karl using identical methodology and key words. A survey of the literature from 2000 through 2003 identified 21 additional studies to supplement the original list of 51. These publications included 8 case reports, 1 prospective cohort study that included some details about thromboembolism among other reported outcomes, 1 prospective cohort study (with case controls) in which thromboembolism was the primary outcome measure, 7 retrospective cohort studies that included some details about thromboembolism among the reported outcomes, and 3 retrospective cohort studies in which thromboembolism was the primary outcome measure, in addition to our study, which was the only one to evaluate the consistent use of a single strategy of prophylactic anticoagulation. Herein are summarized the findings of this now current review of the literature.

## Timing and Incidence of Thromboembolic Complications

Monagle and Karl [45] reviewed eight retrospective studies that had thromboembolic events as the primary outcome measure [16, 17, 23, 32, 36, 42, 54, 59]. The number of patients in these eight separate series ranged from 25 to 654 and totaled 1585. The percentage of patients experiencing thromboses ranged from 3 to 16%, and the percentage experiencing stroke or arterial emboli ranged from 3 to 19%. Among reports published subsequent to that review, Coon and associates [12] investigated the frequency of thrombus in patients followed at the Children's Hospital of Philadelphia after a Fontan operation. Between 1987 and 1999, 592 patients underwent echocardiography after Fontan operations, and 52 patients (8.8%) had intracardiac thrombus. Freedom from thrombus was 92, 90, 84, and 82% at 1, 3, 8, and 10 years after the Fontan operation, respectively. There was no difference in frequency of thrombus based on type of operation (atriopulmonary connection vs lateral tunnel) or the presence of fenestrations. Thrombus was detected in the systemic venous atrium in 26 patients (48%), in the pulmonary venous atrium in 22 patients (44%), in both atria in one patient (2%), in the hypoplastic ventricular cavity in 2 patients (8%), and in the ligated pulmonary artery stump in 1 patient (2%). A cerebral vascular accident was documented at approximately the time of thrombus detection on echocardiography in 8 of the 52 patients (15%). Of these 8, 4 were in atrial fibrillation/flutter and 3 had protein-losing enteropathy. Of the 52 patients with thrombus, 24 (46%) were on low-dose aspirin, 6 (12%) were on warfarin, and 1 (2%) was on heparin (for protein-losing enteropathy) at the time of detection of thrombus. The detection of thrombus on echocardiography was within the first year after Fontan operation in 34 of the 52 cases (65%). The median time interval between Fontan operation and detection of thrombus was 2.3 months (range, 1 day to 163 months). The curve describing the frequency of thrombus occurrence over time closely resembled previously published curves for the development of arrhythmia and protein-losing enteropathy, leading the authors to suggest a lifelong risk of thrombus formation in Fontan patients and the possibility of a codependent relationship between these late complications.

In 2002, Seipelt and associates [56] reported a retrospective series of 101 Fontan operations between 1986 and

1998 with analysis of thromboembolic events as the primary outcome measure. Of 85 survivors available for evaluation, 13 patients (15.3%) experienced thromboembolic events. Type of operation had no influence on the rate of thromboembolism. Patients were further analyzed by medical regimen within three groups: no anticoagulation, aspirin therapy, and Coumadin. There were complex interactions between date of operation, type of operation (modified Fontan vs total cavopulmonary connection), and prophylactic anticoagulant medical regimen (virtually none of the earlier Fontan patients received Coumadin, whereas half of the more recent total cavopulmonary connection patients did). Thromboembolic events occurred in patients within each of the three anticoagulant regimens, but there was a lower incidence in the more recent cohort managed with Coumadin.

Chun and associates [11] reported the incidence of stroke after Fontan procedures in 139 patients as 3.6% (seven strokes in 5 patients). Events occurred between 2 weeks and 9 years postoperatively. Two strokes occurred while on aspirin and Warfarin, two while on aspirin alone, and three while on no anticoagulant medications. Of the 5 patients, 3 had unfenestrated Fontans and 2 had fenestrated Fontans. Curiously, no intracardiac thrombus was detected by transthoracic echo at the time of the strokes. Transesophageal echos were done within a few days of stroke in 2 patients and did not demonstrate intracardiac thrombus. To our knowledge, this is the only study to have identified previous pulmonary artery banding as a risk factor for stroke. The authors invoked a potential mechanism similar to that in patients with a ligated main pulmonary artery stump.

An interesting cross-sectional study evaluating the prevalence of clinically silent pulmonary emboli in adults after Fontan operations was reported in 2003 by Varma et al. [61]. All consecutive adult Fontan patients attending the clinic at the University of Toronto Congenital Cardiac Center for Adults underwent ventilation–perfusion scanning and blood testing for thrombophilic tendency. Five adult patients (17%) had an intermediate or high probability for pulmonary embolism on ventilation perfusion scan, all of which were confirmed by computed tomography (CT) pulmonary angiography. No patient had a thrombophilic tendency (deficiency of protein C, protein S, antithrombin III, or antiphospholipid syndrome), although complete hematologic surveys were not done on patients receiving warfarin. Thirty percent of the patients were taking warfarin because of atrial flutter or atrial fibrillation, either chronic or paroxysmal. None of them had pulmonary emboli. Later age at the time of Fontan operation was associated with increased risk of silent pulmonary embolism, as was the type of Fontan anatomy (lateral tunnel > right atrium–right ventricle connection > atrial pulmonary connection). Not associated with silent pulmonary embolism were atrial arrhythmias, right atrial thrombus, or previous systemic thromboembolic events.

Three studies have compared transthoracic echocardiography (TTE) with transesophageal echocardiography (TEE) in the diagnosis of thromboembolic events after Fontan surgery. Stumper et al. [60], in a cross-sectional survey of 18 patients, found three intracardiac thrombi using TEE, only one of which was detected by TTE. The three positive cases were confirmed by angiography. Fyfe et al. [27] found six thrombi in 4 patients by TEE, only one of which was detected by TTE. The cases defined as positive by TEE were confirmed by angiography, surgery, or resolution of findings after treatment. Balling et al. [7] performed a cross-sectional study of 52 patients after Fontan operations. Seventeen patients (33%) had thrombus seen on TEE, only one of which was identified on TTE. Frequency of thromboembolic events reported is increased in recent studies compared with earlier studies, and the significant incidence of clinically silent thromboembolic events is noteworthy. Improved survival rates, longer duration of follow-up, improved diagnostic studies, and increased awareness of the potential for thromboembolic events must all contribute to the apparent increase in prevalence.

## Mortality Associated with Thromboembolic Complications

In general, information on the management and outcome of thromboembolic events in Fontan patients is scarce and poorly documented. Monagle and Karl [45] summarized the management approaches described in the literature and subsequent outcomes (Table 1).

Complete resolution of thrombosis was obtained in 48% of cases and death occurred in that compendium 25% of cases. Follow-up ranged from 1 month to 5 years [6, 8, 15, 17, 20, 21, 23, 25–27, 29, 32, 35, 36, 38, 39, 41, 50, 54, 58, 60, 62].

## Risk Factors for Thromboembolic Complications

The influence of patient age at operation on subsequent risk of thromboembolic events is uncertain. Whereas at least one cohort study identified older age at Fontan operation as a risk factor [61], other studies showed no correlation between age at surgery and risk of thromboembolic events. In retrospective cohort studies, type of Fontan operation performed, type of material used for the conduit, and the use of valved versus nonvalved conduits did not influence the incidence of venous thrombosis [32, 54]. Potentially

**Table 1** Outcomes of thromboembolic events after Fontan procedures according to antithrombotic treatment[a]

| Treatment | No. treated | Complete resolution | Death | Subsequent embolization, extension, or incomplete resolution | Subsequent takedown of Fontan |
|---|---|---|---|---|---|
| Surgery | 5 | 2 | 2 | 1 | — |
| Surgery + anticoagulation | 14 | 7 | 6 | — | 1 |
| Thrombolysis | 6 | 3 | 1 | 3 | — |
| Thrombolysis + anticoagulation | 12 | 4 | 4 | 8 | — |
| Heparin | 5 | 3 | 1 | 1 | — |
| Coumadin | 23 | 14 | 2 | 7 | 1 |
| Aspirin | 2 | — | — | 2 | — |
| Total | 67 | 33 | 16 | 22 | 2 |

[a] Reproduced from: Monagle and Karl [45]

important exceptions to these general observations are highlighted in a report by Schoof et al. [55] of thrombus development in all three of their patients who underwent extracardiac total cavopulmonary connection using bovine jugular vein (Contegra, Medtronic, Minneapolis, MN, USA) as the extracardiac connection between the inferior vena cava and the pulmonary artery. Although the incidence of thromboembolic events appears to be independent of the individual type of Fontan pathway connections, Konstantinov and associates [47] from the Mayo Clinic have described thrombosis of both intracardiac and extracardiac conduits after modified Fontan operations in patients with azygous continuation of the inferior vena cava. The inference is that conduits carrying only hepatic venous blood may have a higher incidence of thrombosis.

Among potential hemodynamic risk factors for thromboembolic phenomena, low cardiac output, polycythemia, arrhythmias, and right-to-left shunts have all been discussed more completely than they have been analyzed. Although there is a general consensus that right-to-left shunts in congenital heart disease are associated with an increased risk of cerebral vascular embolization and stroke, du Plessis et al. [23] and Day et al. [17] assessed the role of fenestration in causing strokes and neither group found a significantly increased incidence of stroke in patients with fenestrations.

Danielson [16] reported that 16 of 18 patients who had strokes after Fontan operations at the Mayo Clinic had low cardiac output. The number of patients considered to have low cardiac output who did not experience strokes or thromboembolic events is unknown. du Plessis et al. [23] reported that polycythemia had no relationship to the risk of stroke in Fontan patients at Children's Hospital Boston. Rosenthal et al. [54] found arrhythmias in 71% of patients who subsequently developed thrombosis compared to 43% of those who did not. However, 70% of patients diagnosed with thromboembolic events were in sinus rhythm. Already noted was the study by Varma et al. [61], in which patients who received warfarin because of atrial arrhythmias had a zero incidence of clinically silent pulmonary embolism. du Plessis et al. found no relationship between arrhythmia and stroke after Fontan operation. Day et al. [17], in assessing potential risk factors for stroke after the Fontan procedure, reported the presence of residual right-to-left shunts (non-surgical fenestrations) in 6 of 7 patients with neurologic symptoms and in only 3 of 21 asymptomatic patients who also underwent post-Fontan catheterization. However, the denominator of asymptomatic patients with residual shunts is unknown.

In 1997, Kaulitz et al. [36] from Hannover, Germany, analyzed sequelae of the Fontan operation in the 80 survivors among 90 patients who underwent modified Fontan procedures between 1986 and 1994. Of 5 patients (6.2%) in whom intra-atrial thrombus was detected by transthoracic echocardiography, 3 had early postoperative thrombus formation despite heparin therapy. Each was believed to have mild obstruction of the cavopulmonary connection and preoperatively had raised pulmonary arteriolar resistance. Late postoperative atrial thrombosis was diagnosed on routine echocardiogram in 2 patients; both had previously developed signs of protein-losing enteropathy.

## Hematologic Factors in Fontan Patients

Cromme-Dijkhuis et al. [13] were among the first to measure coagulation factors and describe quantitative abnormalities involving both procoagulant and anticoagulant proteins in patients following Fontan operations. In their first study of 37 patients [13], they described 63 coagulation abnormalities in 24 patients. These included subnormal levels of protein C, antithrombin III, and factors II and X. In their second study [14], which evaluated an additional 66 patients, 62% were reported to have protein C deficiency. Deficiencies of protein S (6%), antithrombin III (4%), factor II (36%), factors VII and IX (43%), factor X

(36%), and plasminogen (15%) were also detected. Although these findings have been questioned because the authors did not use age-appropriate reference ranges for normal values [43], they show alterations that suggest the possibility of a procoagulant state after Fontan operations.

Jahangiri et al. [33], in a cross-sectional study of 20 children who had undergone modified Fontan procedures, reported similar coagulation factor abnormalities. However, their findings also included levels of factor VII that were significantly less than the normal range. Factor VII deficiency, if moderate in degree, should predispose to bleeding, not coagulation. This, together with the fact that protein C is a natural anticoagulant synthesized in the liver as a vitamin K-dependent protein, suggests a complex interaction between procoagulant factors and anticoagulant factors in Fontan patients. Furthermore, Jahangiri and other investigators [31] ruled out localized differences in coagulation abnormalities within the heart in patients late after the Fontan operation. Thus, the available information concerning the state of the coagulation system in Fontan patients suggests a complex field of physiologic variables, some potentially predisposing to thrombotic events and some potentially predisposing to a hypocoagulable state. Until recently, no authors had demonstrated any clear relation between the presence of coagulation factor abnormalities and the clinical and hemodynamic condition of the patients. To shed further light on this question, Odegard and colleagues [46] at Harvard designed a prospective case–control study to evaluate coagulation factor abnormalities and hemodynamic variables in children undergoing the Fontan operation. Coagulation factors were assayed in 20 children (age, 6.4 ± 2.9 years) at a mean of 3.7 ± 2.3 years after the Fontan procedure and in 24 age-matched healthy control subjects. Normal reference intervals were based on the control group. Concentrations of protein C, factors II, V, VII, and X, plasminogen, and antithrombin III were significantly lower in Fontan patients compared with age-matched controls. Factor VIII was significantly elevated in 6 patients (35%), 2 of whom had protein-losing enteropathy and thromboembolic events. A higher superior vena cava pressure was predictive of an elevated factor VIII level ($p = 0.003$). No other significant hemodynamic variables were predictive of a procoagulant or anticoagulant abnormality.

Whereas considerable emphasis has been placed on quantitation of coagulation factor proteins in post-Fontan patients, less attention has been focused on the issue of platelet reactivity. Ravn et al. [52] investigated platelet reactivity and quantified coagulation markers in a cross-sectional survey of 24 patients (median age, 11 years) at 2 years (range, 0.5–6) after a total cavopulmonary connection ($n = 14$) or a bidirectional Glenn anastomosis ($n = 10$). The reduction in serum proteins and clotting factors was generally similar to that described by other authors. None of the patients had clinically apparent thromboembolic events. However, increased platelet reactivity was observed *ex vivo* both after collagen-induced platelet aggregation [median, 73% (range, 61–84) in patients vs 61% (range, 47–69) in controls; $p < 0.01$] and after ADP-induced platelet aggregation [median, 69% (range, 52–77) in patients vs 56% (range, 40–66) in controls; $p < 0.05$). Among the many investigators who have examined the influence of connection geometry on the hemodynamic efficiency of various types of Fontan pathways, Monagle and Karl [45] evaluated the role of shear stress, among other major flow parameters, and hypothesized that changes in local flow structure produced changes in maximum shear stress values that may have consequences for platelet activation and thrombus formation in the clinical situation.

## Discussion

Ultimate reduction of the morbidity and mortality associated with Fontan's operation requires a strategy to minimize thromboembolic events. Three decades after the popular acceptance of the modified Fontan procedure as primary therapy, first for tricuspid atresia and later for a wide variety of malformations, there remain more controversies than hard facts concerning the causes and nature of these complications [1, 3, 4, 5, 9, 10, 18, 19, 22, 24]. Although there is general agreement that ligation of the main pulmonary artery, leaving a blind pouch or cul-de-sac distal to the pulmonary valve, is a worrisome substrate for the occurrence of thromboembolism [37, 40, 48, 53], almost any other assertion with respect to thromboembolic events after Fontan operations is the subject of controversy. The extent to which surgical factors and patient factors contribute to the overall risk remains poorly defined. Importantly, there is little agreement regarding the efficacy of various forms of prophylactic anticoagulant therapy in reducing the morbidity and mortality from thromboembolic events after Fontan operations.

What do we know? We know that (1) thromboembolic events occur more frequently, both early and late after modified Fontan operations, than they do after any other form of cardiac reconstruction other than prosthetic valve replacement; (2) thromboembolic events contribute to failure of the Fontan circulation and may occur with increased frequency in the "failing" Fontan circulation; (3) thromboembolic events occur in patients receiving heparin, aspirin, or Coumadin, as well as combinations or none of these; and (4) the factors predisposing to thromboembolic events after Fontan operations likely represent a complex field of biologic factors with multiple interactions. As such, it is very unlikely that a single agent will represent the

solution to this complex problem. In our institution, we completely avoid direct caval cannulation and the use of central venous lines. Single atrial cannulation and a very brief period of hypothermic circulatory arrest to create either a lateral atrial tunnel or extracardiac inferior vena cava-to-pulmonary artery connection avoids dissection around the cavae with the attendant risks of bleeding, caval injury or distortion, and phrenic nerve dysfunction. Obviating the need for repairs at caval cannulation sites, and avoiding the use of central venous lines, minimizes the likelihood of developing a nidus of thrombus within the venous pathway in the perioperative period. The use of only transthoracic atrial lines allows continuous monitoring of cardiac filling pressures during the early postoperative period, without the additional presence of foreign bodies in the cavopulmonary pathway. Inotropic support is routinely administered for 48–72 hours postoperatively, even in patients with ideal hemodynamics. We believe that maximizing cardiac output without significant elevation of venous pressure may contribute to the reduction of early postoperative thromboembolic events.

In our practice, we are encouraged with the results of our initial 5-year trial [30] and subsequent experience over 3 additional years with the use of aspirin (81 mg/day) beginning on the first postoperative day and continuing indefinitely during long-term follow-up. Despite routine surveillance, in addition to focused investigation of all clinically suspicious events, we have detected no thromboembolic events in our patient group. We are aware of the multiple reports of thromboembolic events occurring in Fontan patients while on aspirin, but we have so far not experienced this morbidity in our series.

We emphasize the importance of careful ongoing evaluation of post-Fontan patients for thromboembolic events. Clinically suspicious occurrences must be investigated in a timely fashion. This includes transesophageal echocardiography in circumstances in which there is an alteration from baseline hemodynamics and also transesophageal echocardiography and brain imaging (CT scan and/or magnetic resonance imaging) when there is suspicion of a cerebrovascular event. Investigations described previously have highlighted the occurrence of clinically silent thromboembolic events in patients in other series. Our routine follow-up includes clinical evaluation with transthoracic echocardiography at 6-month intervals and cardiac catheterization with angiography 1 year after the Fontan procedure. A transesophageal echocardiogram is performed in patients who demonstrate new onset of atrial arrhythmias, and both transthoracic echocardiography and cardiac catheterization with angiography are undertaken to investigate any hemodynamic deterioration. Although subclinical thrombi may have been missed in our patients, they have not progressed to clinically relevant events. In addition, although we remain satisfied with the results of this regimen, we concur with those who recommend Coumadin in the setting of poor hemodynamics and chronic venous hypertension and for patients with uncontrolled atrial tachyarrhythmias. We also concur with the use of Coumadin in adult patients who undergo Fontan revisions or conversions for failure of an initial form of Fontan connection.

The problem of reducing thromboembolic complications in Fontan patients is, of course, not as simple as finding a single optimal regimen of anticoagulant therapy. In patients with chronic effusions or protein-losing enteropathy, it is important to measure procoagulant and anticoagulant factor levels. In replacing the protein losses associated with these pathologic conditions, it is important to periodically administer fresh frozen plasma in addition to albumin in order to replete stores of protein C, protein S, and antithrombin III and to avoid a prothrombotic state.

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